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CREATINE METABOLISM

EXCHANGE
FEB 8 1926

A DISSERTATION

Submitted to the Faculty of the Graduate College
of the State University of Iowa
in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy

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EXCHANGE

INTRODUCTION

The data presented in this dissertation represents a continuation of the work previously carried out by the author on creatine metabolism. The first paper[1] was concerned with a comparison of methods for the determination of creatine in the various tissues. The second paper,[2] which served as a basis for a master's thesis, included a series of experiments on the rôle of glycocyamine in creatine formation. The first part of the present investigation is concerned with a study of a series of possible precursors of creatine. The second part is a study of the oxidation of creatine in vivo and in vitro, together with some observations on the conversion of this substance to its anhydride, creatinine.

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PART I

THE ORIGIN OF CREATINE

HISTORICAL

With the appearance of an accurate method for the estimation of creatine and creatinine, investigators early turned their attention to possible precursors of this substance among the protein cleavage products and nitrogenous extractives of various tissues. In spite of the numerous investigations carried out on this subject the origin of this interesting substance is as yet undetermined.

Since the only known cleavage product of protein to contain the guanidine nucleus was the amino acid, arginine, the greater part of the investigations have centered about this substance or its possible intermediate products. Von Hoogenhuyze and Verploegh[3] found no increased elimination of creatine or creatinine in the urine after the ingestion of protein relatively rich in arginine. Jaffe[4] likewise reported negative results after the injection of arginine into rabbits. Inouye[5] believes an increase in creatine occurs when arginine is allowed to incubate with cat liver or is perfused through this organ. Myers and Fine[6] found a very slightly higher muscle content in creatine for rats fed on the arginine rich diet of edestin as compared to controls. Baumann and Marker[7] failed to find any increase of creatine after perfusion of dog's limb or autolysis in vitro. During the past few years Thompson[8] [9] [10] reported numerous injection experiments with arginine on rabbits, ducks and dogs. In most instances he was able to observe an increase in the elimination of creatine in the urine and in some instances an increase of muscle creatine as well. Gibson and Martin[11] found no increased urinary creatine when the arginine rich protein, edestin, was substituted for 80 per cent of the protein of the diet in a case of progressive pseudohypertrophic muscular dystrophy, which quantitatively eliminated ingested creatine. Recently Gross and Steenback[12] have reported that arginine and arginine rich protein given to pigs caused an increased elimination of creatine in

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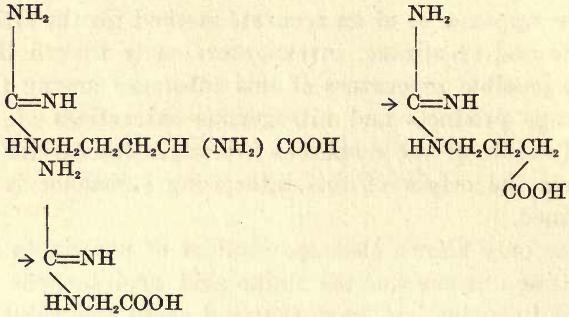
the urine. In our experiments we were unable to obtain decisive results with arginine. To be sure an increase of urinary creatine followed one injection but a similar result was obtained in the control histidine experiment.

If arginine is partly converted into creatine by the animal tissues, glycocyamine may be regarded as an intermediate product in this reaction.



Considerable work has been done on this problem but the question has not been conclusively settled. Czernicki,[13] using the zinc chloride precipitation method, obtained an increase of creatinine in the urine of rabbits after feeding glycocyamine but not when glycocyamidine was fed. Jaffe,[14] using the same method, found that the injection or feeding of glycocyamine led to an increase in the creatine and creatinine fraction both in muscle and urine. Dorner[14] repeated Jaffe's work and employed the more accurate colorimetric method for the determination of creatine and creatinine. He found an increased excretion of creatine after the administration of glycocyamine and also found that muscle in vitro possessed the power to methylate glycocyamine. Mellanby[15] criticizes Jaffe's technique and Dorner's experiments and found no consistent increase in the creative content of chicken muscle after the administration of glycocyamine. More recently Pallidin and Wallenburger[16] have apparently obtained a relatively large increase in creatine when rabbit's muscle was permitted to act on glycocyamine at incubator temperature and also when rabbits were injected with glycocyamine. Gibson and Martin[11] report that feeding glycocyamine to a case of muscular dystrophy resulted in at least 35% being converted to creatine, but conclude that glycocyamine is probably not a stage in ordinary creatine formation. Thompson[17] reports positive results as to creatine content of muscle after injection of glycocyamine into rabbits. Baumann and Hines[2] found no evidence for methylation of glycocyamine in vitro either by perfusion or autolysis. However, the administration of glycocyamine may lead to an increased excretion of creatine in the urine. One experiment in addition to those cited above is reported in this work showing a slight increase in urine creatine following an injection of this substance. Thomas and Goerne[18] obtained negative results with guanido-caproic acid and gamma-methylamino butyric acid.

Volhard[25] synthesized creatine from cyanamide and sarcosine. Jaffe[4] found that glycocoll administered with cyanamide did not neutralize its toxic action. Owing to its toxic effect our injection experiments with cyanamide are inconclusive. Harding and Young[26] have reported that cystine administration intensifies the creatinuria in young dogs. Bauman and Marker[7] report negative results with the perfusion of methyl uredoacetic acid. Riesser[21] [23] studied the effect of injections of choline, betaine and sarcosine on the creatine content of rabbit muscle and urine. This investigator believes that all three substances may produce an increase in the creatine content of rabbit muscle. Thompson[8] however did not observe any additional increase in the creatine elimination when betaine or choline was administered with arginine, nor did the feeding of sarcosine with guandine increase the creatine output. Gibson and Martin[11] reported negative results with sarcosine administration in a case of muscular dystrophy. Recently Shanks[24] reported experiments which tend to show that choline injections increase the content of muscle creatine. Our experiments listed below with these substances do not indicate an increase sufficiently great but what might be accounted for by factors other than a conversion to creatine.

Attempts by Jaffe,[4] Dorner,[14] Pomerenig,[19] and Achelis[20] to show an increased creatine excretion in the urine after the injection of methyl guanidine led to no conclusive results. Riesser[21] also could find no increase in urinary creatine after the injection of either guanidine or methyl guanidine. Thompson[17] reported an experiment in which the administration of guanidine carbonate along with an equal quantity of paraformaldehyde resulted in an increased creatine content of the urine.

Wishart[22] reported that the injection of guanidine salts results in an increase in the content of muscle creatine. Experiments listed below with (alpha) methyl guanido-glyoxalic acid indicate that the injection of this substance may increase urinary creatine. However, this compound is somewhat toxic and the results do not necessarily indicate that it is converted to creatine.

EXPERIMENTAL GLYCOCYAMINE

Experiment A.—A white rabbit weighing 2,160 grams was used for this experiment. The diet consisted of cabbage and oats. The urine was collected in 24 hour periods. Creatine was determined by heating the urine on the water bath with 0.66 N. sulfuric acid for 3 hours. A quantity of glycocyamine greater than that which could be present in the urine during the injection period was added to normal rabbit urine and its effect on the creatine estimation determined. It was found that this quantity of glycocyamine (400 mg.) increased the daily creatine from 10 to 12 mg.

TABLE I

EFFECT OF GLYCOCYAMINE INJECTIONS ON THE COMPOSITION OF URINE

Day	Volume	Creatinine	Creatine	Glycocyamine Injected
	cc.	*mg.*	*mg.*	*mg.*
1	322	82	9	0
2	320	85	24	0
3	322	84	0	0
4	320	93	9	0
5	330	88	9	0
6	354	90	0	0
Average		87	8.5	
7	355	86	24	360
8	335	83	43	360
9	354	90	51	360
10	368	81	58	360
11	345	99	86	0
Average		87.8	52	
*Corrected average		87.8	40	
12	404	76	22	0
13	363	82	16	0
14	368	91	15	0
15	356	82	30	0
Average		82.7	20.7	

*Subtracting the possible increased color value due to glycocyamidine.

ARGININE

Experiment B.—A dog weighing 7 kg. was fed each day with 220 cc. of fresh milk and 160 gm. of dried bread. The urine was obtained by means of a catheter. The bladder was washed with boric acid solution. The arginine was obtained from edestin by the method of Kossel and Kutscher.[27] The pure copper nitrate salt was converted into the carbonate, dissolved in 40 cc. water and neutralized with acetic acid before subcutaneous injection.

TABLE II

SHOWING THE EFFECT OF ARGININE INJECTIONS ON THE COMPOSITION OF URINE

Day	Volume	Creatinine	Creatine*	Total N.	Urea N.	NH$_3$N
	cc.	*mg.*	*mg.*	*gm.*	*gm.*	*gm.*
1	400	180	5	3.889	2.974	0.280
2	400	189	20	3.396	2.791	0.183
3	400	162	14	3.301	2.623	0.190
4	400	178	14	3.578	2.886	0.171
5	410	172	9	3.478	2.758	0.155
6	400	164	25	3.573	2.966	0.166
Average		174	14.5	3.536	2.833	0.191
7†	560	178	14	4.233	3.087	0.210
8†	405	178	4	3.780	3.021	0.194
9	405	191	6	3.456	2.833	0.172
10	410	192	3	3.803	3.133	0.185
Average		185	7	3.818	3.018	0.190

*Creatine expressed as creatinine.

†2 gm. of arginine injected. No toxic symptoms.

Experiment C.—A bitch weighing 7 kg. was fed each day with 220 cc. of fresh milk and 160 gm. of dried bread. The histidine used here as a control was prepared from dried blood essentially as described by Steudel.[28] It was dissolved in 40 cc. of water and neutralized with acetic acid before subcutaneous injection.

TABLE III

EFFECT OF ARGININE AND HISTIDINE INJECTIONS ON THE COMPOSITION OF URINE

Day	Volume	Creatinine	Creatine*	Total N.	Urea N.	NH_2N
	cc.	*mg.*	*mg.*	*gm.*	*gm.*	*gm.*
1	525	167	9	3.801	3.075	0.221
2	500	160	6	3.925	3.217	0.206
3	400	159	2	3.320	2.633	0.149
4	400	160	8	3.708	3.062	0.201
5	400	165	13	3.231	2.655	0.145
6	400	150	6	3.030	2.469	0.104
7	410	165	2	3.542	2.811	0.208
8	400	150	2	3.120	2.562	0.151
9†	400	167	1	2.820	2.278	0.161
10	470	159	15	3.412	2.715	0.222
Average		160.2	6.4	3.391	2.748	0.177
11‡	545	161	9	3.643	2.924	0.181
12‡	400	160	29	3.788	3.157	0.144
13	410	172	11	3.335	2.773	0.146
Average		164	16	3.588	2.951	0.157
14	415	167	5	3.181	2.539	0.148
15	410	175	3	3.375	2.799	0.163
16	410	165	15	3.283	2.802	0.140
17	450	170	13	3.525	2.947	0.210
18	400	169	8	3.153	2.560	0.156
Average		169	9	3.303	2.729	0.163
19§	400	162	13	3.206	2.664	0.144
20§	400	172	15	3.744	3.052	0.179
21	400	171	6	2.772	2.269	0.125
Average		168	11	3.240	2.661	0.149

*Creatine expressed as creatinine.

†The food was not entirely consumed.

‡Injection of 2 gm. of arginine.

§Injection of 1.6 gm. of histidine.

CYANAMIDE

Experiment D.—A bitch weighing 7.3 kg. was placed on a daily diet consisting of 220 cc. of fresh milk and 200 gm. of dried bread. The urine was obtained quantitatively by means of a catheter. The cyanamide was prepared from calcium cyanamide according to the method described by Osterberg and Kendall.[33] The injections were made subcutaneously.

TABLE IV

EFFECT OF THE INJECTION OF CYANAMIDE ON THE COMPOSITION OF THE URINE

Day	Volume	Creatinine	Creatine	Total N.	Urea N.	NH_2N
	cc.	*mg.*	*mg.*	*gm.*	*gm.*	*gm.*
1	400	165	2	3.500		0.189
2	400	164	18	4.088	4.428	0.240
3	415	172	4	3.960	3.324	0.206
4	425	163	25	4.359	3.580	0.198
5	400	167	6	4.438	3.614	0.208
6	400	168	20	4.491	3.740	0.202
7	400	172	8	4.588	3.850	0.182
Average		167	12	4.203	3.589	0.203
8*	550	160	59	4.900	3.950	0.168
9†	400	143	61	4.200	3.384	0.280
10†	470	163	20	4.717	3.860	0.293
11†	400	160	22	4.438	3.388	0.252
12†	400	165	8	4.340		
13†	400	165	21	4.500		
14†	485	173	12	4.331		
Average		161	29	4.489		

*Injected 150 mg. of cyanamide. This was followed by vomiting and other toxic symptoms.

†Injected 50 mg. of cyanamide. Even the smaller doses exerted a toxic effect, as manifested by anorexia and occasional vomiting.

(ALPHA)-METHYLGUANIDO GLYOXALIC ACID

Experiment E.—A bitch weighing 7 kg. was placed on a diet of fresh milk and dried bread. The urine was collected quantitatively by means of a catheter. The (alpha) methylguanido-glyoxalic acid was prepared by the action of mercuric acetate on creatine, according to the method described by Baumann and Ingvaldsen.[29]

TABLE V

EFFECT OF INJECTION OF (ALPHA) METHYLGUANIDO-GLYOXALIC ACID ON THE COMPOSITION OF THE URINE

Day	Volume	Creatinine	Creatine*	Total N.	Urea N.	NH_2N
	cc.	*mg.*	*mg.*	*gm.*	*gm.*	*gm.*
1	400	172	16	3.547	2.942	0.210
2	410	165	15	3.659	3.201	0.186
3†						
4†	630	172	30	3.144	2.646	0.106
5†	425	180	144	3.258	2.673	0.183
6	410	174	17	2.727		
7	545	183	3			
8	470	174	4			
11†	400	177	3			
12†	400	168	5			
13†	435	180	9			
14†	545	180	6			
15†	620	172	17			

*Expressed as creatine.

†From 0.1750 to 0.4000 gm. of a-methylguanido-glyoxylic acid were injected each day.

Experiment F.—A bitch weighing 8 kg. was placed on a daily diet of 250 cc. fresh milk and 160 gm. of dried bread.

TABLE VI

EFFECT OF SUBCUTANEOUS INJECTION OF (ALPHA) METHYLGUANIDO-GLYOXALIC ON THE COMPOSITION OF THE URINE

Day	Volume	Creatinine	Creatine	Total Nitrogen
	cc.	*mg.*	*mg.*	*gm.*
1	690	220	5	3.79
2	655	215	4	3.59
3	670	205	10	3.49
4	600	205	12	3.49
5	650	209	12	3.54
6	500	203	4	3.46
7	730	206	0	3.55
Average	...	209	6.7	3.56
8*	750	218	8	3.65
9	590	215	19	3.76
10	620	217	8	3.52
Average	...	217	11	3.64

*Injected 300 mg. (alpha) methylguanido-glyoxalic acid and dissolved in physiological saline solution.

Experiment G.—A bitch weighing 10 kg. was placed on a diet of powdered milk, dried bread and water. The (alpha) methylguanido-glyoxalic acid was dissolved in physiological saline solution and injected into the femoral vein under light ether anesthesia.

TABLE VII

EFFECT OF INTRAVENIOUS INJECTION OF (ALPHA) METHYLGUANIDO-GLYOXALIC ACID ON THE COMPOSITION OF THE URINE

Day	Creatinine	Creatine
	mgs.	*mgs.*
1	464	7
2	430	20
3	443	18
4	450	17
5	423	16
6	434	14
7	465	27
Average	444	17
8*	466	115
9	429	12

*Injected 600 mgs. of (alpha) methylguanido-glyoxalic acid.

IN VITRO AUTOLYSIS OF DOG'S MUSCLE AND (ALPHA) GUANIDO-GLYOXALIC ACID

Experiment H.—Freshly hashed dog muscle was weighed into a flask containing (alpha) methylguanido-glyoxalic acid dissolved in Henderson's phosphate solution. After the addition of toluene the flask was placed in an incubator from 40 to 45 hours and the creatine content determined according to the method of Janney and Blatherwick.[30] The controls which had remained in the incubator were analyzed according to the same method.

TABLE VIII

Weight of Muscle	Time C 38° C	Methylgyanido-Glyoxalic Acid	Creatine	Weight of Muscle	Time C 38° C	Methylgyanido-Glyoxalic Acid	Creatine
gms.	*hrs.*	*mgs.*	*per cent*	*gms.*	*hrs.*	*mgs.*	*per cent*
Experiment				Control			
5.559	40	25	.362	5.713	40	0	.342
4.524	40	25	.363	5.765	45	0	.353
5.354	45	25	.355				
4.949	45	25	.356				
Average			.359				.3475

TOXICITY OF (ALPHA) METHYLGUANIDO-GLYOXALIC ACID

Experiment I.—150 mg. of (alpha) methylguanido-glyoxalic acid were dissolved in physiological saline and injected intraperitonealy into a guinea pig, weighing 350 gm., produced tonic contractions, stupor and death within two hours.

SARCOSINE

Sarcosine with urea was perfused four times through the muscle of dogs. The technique of perfusion† was that described by Baumann and Marker.[7] The creatine content of liver or muscle in the following experiments is expressed in terms of mg. per gm. of dry tissue. The creatine in muscle was determined on each sample* according to the methods of Janney and Blatherwick[30] and Baumann.[31] Creatine in the blood and pefusates was determined by a modification of this method, described by Baumann and Marker.[7] The water content of muscle was determined by noting the loss after drying to constant weight in vacuo over concentrated sulphuric and phosphorus pentoxide at room temperature. The sarcosine was prepared according to Baumann.[32]

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†We are indebted to Dr. J. I. Marker for his assistance with the perfusion experiments.

*A comparison of these two methods has been reported in a paper by Baumann and Hines.[7]

Experiment J.—The leg of a dog weighing 14.5 kg. was perfused with defibrinated blood containing 4 gm. of sarcosine and 4 gm. of urea for 1 hour. The entire limb weighed 2,625 gm. while the muscle weighed 1,250 gm.

	Perfusate		Muscle	
	Volume	Creatine	Water	Creatine
	cc.	*mg. percent*	*percent*	*mg. per gm.*
Unperfused control	225	12.3	73.83	16.01
After perfusion	255	10.6	72.07	15.50

Experiment K.—The leg of a dog weighing 27 kg. was perfused for 1 hour with a mixture containing 30 cc. of 0.9 per cent sodium chloride solution, 4 gm. of sarcosine, 2 gm. of urea, and 250 cc. of defibrinated dog's blood. The entire leg weighed 1,930 gm. and its muscular tissue 1,135 gm.

	Perfusate		Muscle	
	Volume	Creatine	Water	Creatine
	cc.	*mg. percent*	*percent*	*mg. per gm.*
Unperfused control	280	12.6	67.48	11.75
After perfusion	200	12.3	66.42	11.40

Experiment L.—The leg of a dog weighing 9.09 kg. was perfused for 1 hour with a solution containing 2 gm. of sarcosine, 2 gm. of urea, 25 cc. of 0.9 per cent Na Cl solution, and 255 cc. of defibrinated blood. The leg weighed 682 gm. and its muscular tissue weighed 454 gm.

	Perfusate		Muscle	
	Volume	Creatine	Water	Creatine
	cc.	*mg. percent*	*percent*	*mg. per gm.*
Unperfused control	250	11.0	71.42	12.61
After perfusion	250	10.6	72.39	12.87

Experiment M.—The hind limb of a dog weighing 15.9 kg. was perfused with defibrinated blood containing 3 gm. of sarcosine and 3 gm. of urea for 1 hour.

	Perfusate		Muscle	
	Volume	Creatine	Water	Creatine
	cc.	*mg. percent*	*percent*	*mg. per gm.*
Unperfused control	175	15.1	72.82	13.17
After perfusion	225	12.7	74.46	14.44

SUMMARY OF EXPERIMENTS ON PERFUSION OF MUSCLE WITH SARCOSINE AND UREA

	Unperfused Control		After Perfusion	
No.	Creatine in Total Perfusate	Creatine in Muscle	Creatine in Total Perfuse	Creatine in Muscle
	mg.	*mg. per gm.*	*mg.*	*mg. per gm.*
Experiment J	27.7	16.01	27.0	15.50
Experiment K	35.3	11.75	34.4	11.40
Experiment L	25.5	12.61	26.5	12.87
Experiment M	26.3	13.17	28.6	14.44
Average	28.7	13.385	29.1	13.552

BETAINE

Experiment O.—The hind leg of a dog weighing 24.1 kg. was perfused for 1 hour with a mixture of 200 cc. of defibrinated blood, 25 cc. of 0.9 per cent sodium chloride solution, 3 gm. of betaine hydrochloride, and 3 gm. of urea. The betaine and urea were dissolved in the salt solution and neutralized with sodium hydroxide using phenolphthalein as an indicator. The total weight of the limb was 2,273 gm., that of the muscle alone was 1,477 gm.

	Perfusate		Muscle	
	Volume	Creatine	Water	Creatine
	cc.	*mg. percent*	*percent*	*mg. per gm.*
Unperfused control	225	14.3	73.47	14.02
After perfusion	230	14.1	73.40	44.10

CHOLINE

Experiment P.—The hind leg of a dog weighing 19.5 kg. was perfused for 1 hour, with defibrinated blood, to which portions of a neutralized solution of choline chloride and urea (each 3 gm.) were added from time to time. The choline was prepared from the lecithin of egg yolk.

	Perfusate		Muscle	
	Volume	Creatine	Water	Creatine
	cc.	*mg. percent*	*percent*	*mg. per gm.*
Unperfused control	275	10.6	72.78	14.84
After perfusion	300	11.5	73.78	14.68

Experiment Q.—The hind leg of a dog weighing 24.5 kg. was perfused for 1 hour with defibrinated blood to which were added 40 cc. of a neutralized aqueous solution of choline chloride (3 gm.) and urea (4 gm.) as in the previous experiment.

	Perfusate		Muscle	
	Volume	Creatine	Water	Creatine
	cc.	*mg. percent*	*percent*	*mg. per gm.*
Unperfused control	265	13.2	73.34	14.10
After perfusion	220	11.2	72.98	14.54

Experiment R.—The hind leg of a dog weighing 29.3 kg. was perfused for 1 hour with defibrinated blood, to which were added from time to time small quantities of a neutralized solution of choline chloride (3 gm.) and urea (3 gm.) dissolved in 25 cc. of water.

	Perfusate		Muscle	
	Volume	Creatine	Water	Creatine
	cc.	*mg. percent*	*percent*	*mg. per gm.*
Unperfused control		13.4	71.01	11.93
After perfusion		13.5	71.18	12.52

Experiment S.—The hind leg of a dog weighing 8.4 kg. was perfused for 1 hour with defibrinated blood to which small quantities of a neutralized solution of choline chloride (3 gm.) and urea (3 gm.) in 25 cc. of water were added from time to time. The weight of the entire limb was 625 gm., that of the muscle alone 400 gm.

	Perfusate		Muscle	
	Volume	Creatine	Water	Creatine
	cc.	*mg. percent*	*percent*	*mg. per gm.*
Unperfused control	180	13.4	70.90	13.16
After perfusion	210	11.3	71.98	13.77

SUMMARY OF EXPERIMENTS ON PERFUSION OF MUSCLE WITH CHOLINE AND UREA

	Unperfused Control		After Perfusion	
No.	Creatine in Total Perfusate	Creatine in Muscle	Creatine in Total Perfusate	Creatine in Muscle
	mg.	*mg. per gm.*	*mg.*	*mg. per gm.*
Experiment P	29.2	14.84	28.6	14.68
Experiment Q	35.0	14.10	24.6	14.54
Experiment R		11.93		12.52
Experiment S	29.4	13.16	23.7	13.77
Average	29.4	13.508	25.6	13.878

DISCUSSION

No trustworthy conclusions can be drawn from a single experiment with glycocyamine. However, when those previously reported by the author are considered, it appears that the injection of this substance leads to an increase in the urinary creatine, as determined by the colorimetric method. In the case of autolysis and perfusion experiments no increase in creatine was found provided proper corrections were made for the color value due to the anhydride of glycocyamine, formed during the process of converting creatine to creatinine. However, when this correction is applied to the urine, it still leaves an appreciable increase in the creatine of the urine over the control periods. We believe the rather large increases of creatine obtained by other authors with autolysis of muscle and glycocyamine can be accounted for by their failure to properly control the color due to glycocyamidine. Since glycocyamine has never been isolated from animal tissues and only negative results obtained from perfusion and autolysis experiments, the increased urinary creatine following its administration cannot be considered as proof that this substance is methylated in the body to form creatine.

Our experiments with arginine do not offer any evidence that this substance is a percursor of creatine. In one experiment (Exp. B) the average daily excretion of creatine during the injection period was even less than during the control days. In another experiment (Exp. C) a slight increase in the creatine fraction of the urine was observed. However, the injection of the amino acid, histidine, as a control, also led to a slight increased output of creatine in the urine. These results are in keeping with those obtained by Baumann and Marker in this laboratory.

The increased output of creatine in the urine following the injection of cyanamide (Exp. D) cannot be considered, in any way, as evidence that this substance is converted to creatine in the animal organism. The substance is quite toxic and the disturbances resulting from this may easily account for the increased urinary creatine. The same explanation may apply to our experiments with (alpha) methylguanido-glyoxalic acid as it is also somewhat toxic. This substance was selected for study with the idea that it might be slowly decomposed in the tissues to form free methyl guanidine rather than undergo reduction.

The perfusion experiments listed above with sarcosine and betaine fail to cause any increase in the creatine content of muscle tissue. The four experiments on the perfusion of choline and urea show that the perfused limb to have a slightly higher content of creatine than the control unperfused limb when expressed in terms of mg. per gm. of dry tissue. However, the increase is slight and considering the marked pharmacological action of choline they cannot be considered as proof that this substance is converted to creatine.

In spite of the fact that several of the above named substances may at times, under the various experimental procedures, lead to an increase in the creatine content of muscle or urine, we believe that these effects may be accounted for by toxic action and are not proof that these substances are converted to creatine in the animal body.

CONCLUSIONS

1. Injection, autolysis and perfusion experiments were made with glycocyamine, arginine, histidine, sarcosine, betaine, choline, cyanamide and (alpha) methyl guanido-glyoxalic acid and their effects upon the creatine content of muscle and urine in dogs and rabbits were determined.
2. The increase obtained at times in the creatine content of muscle and urine, we believe, might be accounted for by other reasons than a conversion of any of the substances used as such to creatine in the animal body.
3. The results obtained do not warrant the conclusion that any of the above named substances are precursors of creatine.

BIBLIOGRAPHY

1. Baumann, L., and Hines, H. M., *J. Biol. Chem.*, 1916, XXIV, 439.
2. Baumann, L., and Hines, H. M., *J. Biol. Chem.*, 1917, XXXI, 549.
3. Von Hoogenhuyze, C. J. C., and Verploegh, H., *Z. Physiol. Chem.*, 1908, LVII, 161.
4. Jaffe, M., *Z. Physiol. Chem.*, 1906, XLVIII, 430.
5. Inouye, K., *Z. Physiol. Chem.*, 1912, I XXXI, 71.
6. Myers, V. C., and Fine, M. S., *J. Biol. Chem.*, 1915, XXI, 389.
7. Baumann, L., and Marker, J., *J. Biol. Chem.*, 1915, XXII, 49.
8. Thompson, W. H., *J. Physiol.*, 1917, LI, 347.
9. Thompson, W. H., *J. Physiol.*, 1917, LI, 11.
10. Thompson, W. H., *J. Physiol.*, 1917, LI, 111.

11. Gibson, R. B., and Martin, F. T., *J. Biol. Chem.*, 1921, XLIX, 319.
12. Gross, E. G., and Steenbock, H., *J. Biol. Chem.*, 1921, XLVII, 33.
13. Czernicki, W., *Z. Physiol. Chem.*, 1905, XLIV, 294.
14. Dorner, G., *Z. Physiol. Chem.*, 1907, LII, 225.
15. Mellanby, E., *J. Physiol.*, 1908, XXXVI, 447.
16. Palladin, A., and Wallenburger, L., *Compt. rend. Soc. Biol.*, 1915, XXVIII, 111.
17. Thompson, W. H., *Biochem. J.*, 1917, XI, 307.
18. Thomas, K., and Goerne, M. G. H., *Z. Physiol. Chem.*, 1918-19, CIV, 73.
19. Pommerenig, *Beitrage*, 1901, I, 561.
20. Achelis, W., *Z. Physiol. Chem.*, 1906, L, 10.
21. Riesser, O., *Z. Physiol. Chem.*, 1914, XC, 221.
22. Wishart, G. M., *J. Physiol.*, 1920, LIII, 440.
23. Riesser, O., *Z. Physiol. Chem.*, 1913, XXXVI, 415.
24. Shanks, W. F., *P. Physiol.*, 1921, LV, p. viii.
25. Volhard, J., *Sitzungsb. koenigl. bayer. Akad. Wissensch.*, 1868, II, 472.
26. Harding, V. J., and Young, E. G., *J. Biol. Chem.*, 1920, XLI, p. xxxvi.
27. Kossel, A., and Kutscher, F., *Z. Physiol. Chem.*, 1900-01, XXXI, 165.
28. Steudel, H., in Abderholden, E., *Handl. Biochem. Arbeitsmethoden*, Berlin and Vienna, 1910, II, 505.
29. Baumann, L., and Ingvaldsen, T., *J. Biol. Chem.*, 1918, XXXV, 15.
30. Janney, N. W., and Blatherwick, N. R., *J. Biol. Chem.*, 1915, XXI, 567.
31. Baumann, L., *J. Biol. Chem.*, 1914, XVII, 15.
32. Baumann, L., *J. Biol. Chem.*, 1915, XXI, 563.
33. Osterberg, A. E., and Kendall, E. C., *J. Biol. Chem.*, 1917, XXXII, 297.

Part II

CONCERNING THE DESTRUCTION OF CREATINE IN VIVO AND VITRO

INTRODUCTION

At the beginning of this investigation it was planned to study the relationship of creatine metabolism to the methyl guanidine content of normal tissues and the occurrence of this body in the urine and blood in conditions of parathyroid insufficiency in animals. An investigation of the methods, used to determine methyl guanidine in the presence of creatine or creatinine in tissue extracts, blood, and urine by previous investigators, has convinced the author that these methods are unsuitable for any analytical investigation. Work on this problem has been postponed until more reliable methods are available for the determination of guanidine compounds. An investigation has been made of the possibility that a relationship exists between creatine and carbohydrate metabolism, analogous to the ketogenic and antiketogenic balance, existing in the oxidation of fat and carbohydrate. The effect of variations in the amount of fat and carbohydrate in the diets upon the creatine has been studied in two cases of well established creatinuria. The effect of glucose upon the oxidation of creatine by hydrogen peroxide has been observed. A study of the effect of the addition of pancreas extract to autolyzing muscle on the sugar content and the creatine-creatinine balance has been made. The effect of kidney extract has been compared to muscle extract in its ability to convert creatine to creatinine in vitro.

HISTORICAL

Our detailed knowledge of creatine dates from Liebig's[1] classical investigation of the constituents of muscle juice in 1847. Liebig isolated creatine from the flesh of different animals and converted it to its anhydride, creatinine, and found it to be identical with a substance isolated from urine by Pettenkofer[2] in 1844. Our knowledge of the physiological significance of creatine and creatinine made little progress until Folin[3] [4] in 1904 published an accurate and convenient method for their estimation in biological fluids, and this, together with his theory of metabolism, has led during recent years to many investigations on the physiology of creatine and creatinine.

Folin's[4] denial of a genetic relationship between creatine and creatinine has not met with general acceptance. Most observers hold that creatinine is derived in some manner from creatine. According to Mellanby,[5] however, the reverse is true. He believes that creatinine is formed in the liver from substances brought there by the blood stream and is subsequently rendered innocuous by hydration to creatine. Myers and Fine[6] point out the interesting fact that, although the creatine content of muscle varies from species to species, it is remarkably constant in the individuals of the same species and that those having the greatest creatinine output in the urine also have the highest muscle content of creatine. These authors[7] in a subsequent paper present experiments to show that incubated muscle, liver and blood bring about the conversion of creatine to creatinine in amounts greater than can be accounted for by the hydrogen ion concentration of the medium. Hammett[8] has, likewise, made a study of this transformation. Recently, Behre and Benedict[9] have reported that blood contains only creatine and that the creatinine figures previously reported are due to the inadequacy of the available analytical methods. They suggest that possibly the kidney is the seat of the transformation of creatine to creatinine. The experiments carried out in connection with this work indicate that autolysis of kidney tissue leads to no greater conversion of creatine to creatinine than does muscle tissue alone under the same conditions.

Although little is known as to the origin and significance of the creatinine of the urine, most experimenters agree that the amount of this substance is independent of the protein intake but is dependent upon body weight and muscular development. However, no such harmony of opinion exists as to the factors which are responsible for the occurrence of creatine in the urine. The most common conditions under which creatine excretion has been noted in man are simple starvation, diabetes, childhood, pregnancy, fevers, hyperthyroidism and certain diseases involving muscular atrophy. As to the factors which cause the appearance of creatine in the urine in the above named conditions, opinions are many and diverse. According to Denis[10] most of these conditions are accompanied by a high level of protein metabolism. Folin and Denis[11] believe that the creatine in children's urine is exogenous in origin and is due to an excessive high level of protein consumption in proportion to mass of muscles in the body. A similar conclusion was arrived at by Denis and Krammer.[12] Recently Gamble and Goldschmidt[13] have reported experiments which suggest that the preformed creatine in the ingested cow's milk may be an important factor in the creatinuria of children. Rose, Dimmitt and Bartlett[14] found that the ingestion of diets high in protein failed to induce the excretion of creatine and conclude that there is no evidence for an exogenous origin of creatine. Denis and Minot[15] claim, however, that a very high nitrogen intake in the form of protein may cause creatinuria in women but not in men. Denis,[10] in a study of five cases of Graves disease, found that the amount of creatine excreted is dependent upon the intake of protein. Recently, Gross and Steenbock,[16] from a study of the creatine elimination when sheep's thyroid was fed to pigs, suggest that creatine formation is primarily dependent upon the balance that obtains between the arginase and the oxidative systems whereby arginine is destroyed; the thyroid principle accelerates the latter at the expense of the former. One observation in the course of this investigation confirms the results obtained by Gibson and Martin[17] in that the replacement of part of the protein of the diet by gelatin may increase the output of creatine in children with muscular dystrophy.

The carbohydrate deficiency theory as a cause of creatinuria has held a prominent place in the literature. Cathcart[18] reported that creatine excretion induced in men by fasting could be reduced to nil by the administration of a carbohydrate diet. Mendel and Rose[19] confirmed these results with rabbits. Rose[20] suggests that creatinuria in children is due to the fact that the cells are in a state of partial carbohydrate hunger because of the excessive demand for carbohydrates. The same author[21] in a later paper concludes that the behavior of creatine elimination in phloridzin and pancreatic diabetes offers additional and conclusive proof of the dependence of creatine elimination upon carbohydrate utilization. Myers and Fine[22] believe that the decreased elimination of creatine after feeding of carbohydrate is dependent upon the sparing action of carbohydrate on protein metabolism. Underhill and Baumann[23] believe that a carbohydrate deficiency in the absence of acidosis may cause the elimination of creatine. Benedict and Osterberg have reported experiments in which they were able to keep a dog, rendered completely diabetic with phloridzin, in nitrogen equilibrium by high protein feeding and in spite of the marked elimination of creatine during this period, an analysis of the muscle showed no decrease in the content of creatine as compared to the normal. From these experiments they conclude that creatine elimination is not an index of cellular destruction and that, normally, large amounts of it are destroyed in the body.

Since carbohydrate insufficiency is often accompanied by acidosis, it is interesting to note that the relationship of the latter condition to creatinuria has received the attention of numerous investigators. Krause and Cramer[25] pointed out that creatinuria occurs in all conditions which lead to an acidosis. They cited chiefly the creatinuria of starvation and diabetes. Underhill[26] showed that an acid producing diet led to an increased output of creatine in rabbits and that the creatine of the urine was diminished or absent on a diet producing an alkaline urine. Sawyer, Stevens and Baumann[27] observed a marked increase in the excretion of creatine in children during the period of acidosis, produced by low calory diets or diets containing high fat and low carbohydrate. These authors express their belief, however, that acidosis as such is not the cause of the creatinuria. Gamble and Goldschmidt[13] have found that the administration of sodium bicarbonate or dilute hydrochloric in diets of children are without effect on the creatine output.

In the experimental part of this investigation it was planned to ascertain whether a relationship existed between carbohydrate metabolism and the formation and subsequent elimination of creatine analogous to that reported by Shaffer[28] [29] [30] for carbohydrate metabolism and acetone formation and elimination. When the proportion of acetoacetic acid to glucose was that of 1 molecule of acetoacetic acid to 1 of glucose, the former substance was almost completely oxidized. When the proportion of glucose was less or entirely absent, a considerable portion of the acetoacetic escaped oxidation. The experiments reported here on the oxidation of creatine in the presence of glucose by an alkaline solution of hydrogen peroxide would indicate that this substance at least, has little effect on the rate of the oxidation of creatine under the experimental conditions tried. Two cases of well established creatinuria are studied as to the effect of variations in the relative amounts of fat and carbohydrate upon the creatine output and the fate of ingested creatine. The results indicate that no such relationship exists between creatine destruction and carbohydrate metabolism analogous to that reported by Shaffer for the acetone formation and carbohydrate metabolism. This conclusion is further confirmed by an experiment carried out in connection with other work which shows that a dog, rendered completely diabetic with phloridzin, failed to eliminate any greater percentage of ingested creatine than was found by other investigators[17 13 31 32] in conditions with much less or no derangement of carbohydrate metabolism. In view of the recent work by Banting and Best[33] on the preparation of extracts of the pancreas which are able to lower the hyperglycemia and lessen or prevent the glycosuria in depancreatized dogs, it was deemed advisable to repeat the experiments of Cohnheim[34] on the effect of added pancreas extract to autolyzing muscle on the disappearance of reducing sugar and to investigate whether an extract of pancreas prepared in this manner would have an effect on the creatine and creatinine balance of the autolyzing mixture. Levene and Meyer[34] some years ago repeated Cohnheim's experiments and showed that the disappearance of reducing sugar under these conditions was due not to an oxidation but to a polymerization reaction. The latter authors, however, employed a boiled extract of the pancreas and Banting and Best[33] have shown that boiling destroys the active principle of the pancreas as judged by its effect on relieving glycosuria and hyperglycemia in depancreatized dogs. The experience of the author is that pancreatic extrants prepared according to Banting and Best[33] do not lead to an oxidation of sugar or to any effect on the creatine and creatinine balance of autolyzing muscle.

EXPERIMENTAL

AUTOLYSIS OF MUSCLE AND PANCREAS EXTRACTS

Experiment A.—100 gm. of fresh dog muscle was hashed and ground in a mortar with fine sand. To prevent bacterial activity the preparation was mixed with toluol and chloroform in Henderson's phosphate solution. The fluid was expressed through cloth and the residue subjected to pressure in a Buchner press. To the combined fluids 4 grams of glucose was added and the total made to a volume of 200 cc. The pancreas extract was prepared according to the method described by Best and Banting.[33] The dry resin-like residue obtained from evaporation of the acid alcohol extract was emulsified with 25 cc. Tyrode's solution. 10 cc. portions of the muscle extract were added to a series of test tubes. To some of these tubes was added 1 cc. of pancreas extract, while others served as a control. After the addition of toluol and chloroform, the tubes were incubated for periods of 22 to 44 hours. After which they were analyzed for sugar, preformed creatinine and total creatinine. The sugar determinations were carried out according to thee method of Folin and Wu,[35] after heating in boiling water bath with 1% Hcl for 75 minutes to hydrolize any complex sugars present. Previous to this determination the acid solutions were neutralized with Na OH, using phenolpthalein as an indicator, and dilutions made so that the concentration was suitable for the usual blood sugar standards. The preformed creatinine was determined according to the method described by Folin and Wu [35] for creatinine in blood. Total creatinine was determined, after conversion with normal hydrochloric acid in boiling water bath for 2½ hours, in the usual manner.

THE EFFECT OF THE ADDITION OF PANCREAS EXTRACT ON THE CONTENT OF SUGAR, PREFORMED CREATININE AND TOTAL CREATINE IN AUTOLYZING MUSCLE EXTRACT

TABLE I

Pancreas Extract Added	Time of Incubation	Preformed Creatinine	Total Creatine*	Total Sugar
cc.	*hrs.*	*mg. per 10 cc.*	*mg. per 10 cc.*	*mg. per 10 cc.*
0	0	0.322	10.00	223.9
1	0	0.312	9.89	213.8
Average		0.317	9.94	218.8
0	44	0.251	9.66	206.8
0	44	0.465	9.27	203.6
0	44	0.330	9.54	203.0
0	44	0.251	9.72	202.1
0	22	0.400	9.35	222.0
0	22	0.665	9.95	226.7
Average		0.394	9.58	210.7
1	22	0.565	9.45	214.8
1	44	0.280	9.60	220.0
1	44	0.500	9.54	203.6
1	44	0.525	lost	205.2
1	44	0.300	9.58	211.5
1	22	0.665	9.95	226.2
Average		0.472	9.62	213.5

*Expressed as creatinine.

THE EFFECT OF KIDNEY EXTRACT ON THE CONVERSION OF CREATINE TO CREATININE DURING INCUBATION

Experiment B.—100 gms. of fresh ox kidney was hashed and ground with fine sand in a mortar. After the addition of Tyrode's solution, the juice was expressed as far as possible through cloth and the residue subjected to pressure with a Buchner press. All liquids were combined and after the addition of 200 mg. creatine made up to a volume of 200 cc. A preparation of rabbit muscle was made according to the above method for kidney with the exception that no creatine was added. To a series of graduated centrifuge tubes 5 cc. portions were added, diluted to 10 cc. with Tyrode's or Henderson's phosphate and allowed to incubate from 20–40 hours with chloroform and toluol. The preformed creatinine was determined according to a modified Folin and Wu method; described by Hammett.[36] Controls were carried out on extracts without incubation.

THE EFFECT OF KIDNEY EXTRACT ON THE CONVERSION OF CREATINE TO CREATININE DURING INCUBATION

Experiment C.—Both kidneys from a dog, killed by hemorrhage, were quickly pealed, hashed and ground with fine sand in a mortar. The expressed liquids were diluted to a volume of 200 cc. with Tyrode's solution after the addition of 200 mg. of creatine. To each of six test tubes 20 cc. portions of the above extract was added. 20 cc. of Henderson's phosphate solution was added to three tubes and 20 cc. of Tyrode's solution to the others. After the addition of toluol and chloroform to retard bacterial action, all tubes were incubated for 46 hours at 37° C. except those serving as a control. Preformed creatinine was determined according to the modified Folin and Wu method described by Hammett.[36]

TABLE II

THE EFFECT OF KIDNEY EXTRACT ON THE CONVERSION OF CREATINE TO CREATININE DURING INCUBATION

	Control	Incubated					
		2½ cc. Muscle Extract			2½ cc. Kidney Extract		
		Tyrode's (5 cc.)			Henderson's Phosphate Solution (5 cc.)		
	Mg. per 100 cc.	Time	Creatinine	Percent Increase	Time	Creatinine	Percent Increase
		hrs.	*mg. per 100 cc.*		*hrs.*	*mg. per 100 cc.*	
	2.16	20	3.24	48	20	3.08	41
	2.22	40	4.20	92	40	3.48	59
Average	2.19		3.72	70		3.28	50
		5 cc. Muscle Extract					
	2.76	20	3.90	47	20	3.88	46
	2.56	40	6.00	126	40	4.70	76
Average	2.66		4.95	86		4.29	61
		5 cc. Kidney Extract					
	1.88	20	3.00	83	20	3.10	88
	1.40	40	3.14	87	40	3.42	108
Average	1.64		3.07	87		3.26	98

TABLE III

CHANGES IN THE CREATININE CONTENT ON INCUBATION WITH KIDNEY EXTRACT

	Control	Incubated 20 cc. Kidney Extract		
	Mg. per 100 cc.	Mg. per 100 cc.	Percent Increase	Solution Added
	0.75	1.39	63	20 cc. Henderson's Phosphate
	0.95	1.26	48	20 cc. Henderson's Phosphate
		1.87	120	20 cc. Tyrode's
Average	0.85	1.51	77	

THE EFFECT OF ADDED GLUCOSE ON THE OXIDATION OF CREATINE BY HYDROGEN PEROXIDE IN 1% SODIUM HYDROXIDE SOLUTION

Experiment D.—After standing the designated time the tubes were heated 3 hours in boiling water bath with normal HCl., then evaporated to dryness to expel any remaining H_2O_2. The creatine content was then determined colorimetricly.

TABLE IV

Temp.	Time	Millimoles Creatine	Millimoles Glucose	Millimoles Hydrogen Peroxide	Creatine	Percent Lost
	hrs.				*mg.*	
	0	10	0	0	23.8	0
	0	10	10	20	23.8	0
25° C.	18	10	10	20	17.6	26
25° C.	24	10	10	20	17.8	25
25° C.	40	10	10	20	17.1	24
37° C.	18	10	10	20	17.1	24
37° C.	24	10	10	20	16.5	31
	0	10	0	20	24.3	0
25	24	10	0	20	21.7	9
25	40	10	0	20	22.0	8
25	18	10	0	20	21.7	9
37	18	10	0	20	19.8	17

TABLE V

37° C.

Time Hrs.	Millimoles Creatine	Millimoles Glucose	Millimoles H_2O_2	Creatine Mg.	Percent Lost
	10	0	0	10.8	
2	10	10	80	7.64	29.2
24	10	10	80	6.35	41.2
40	10	10	80	5.75	50.9
64	10	10	80	3.75	69.4
88	10	10	80	2.84	73.7
2	10	0	80	9.50	12.0
24	10	0	80	7.08	34.4
40	10	0	80	6.75	37.5
64	10	0	80	5.28	51.1
88	10	0	80	4.68	56.7
2	10	0	0	9.50	12.0
40	10	0	0	9.40	12.9
64	10	0	0	7.20	33.3
88	10	0	0	6.19	42.7

Experiment E.—Oxidation of creatine by hydrogen peroxide in Henderson's phosphate solution, Ph. 7.4, with and without the addition of glucose.

TABLE VI

Temp.	Time	Millimoles Creatine	Millimoles Glucose	Millimoles H_2O_2	Creatine	Percent Lost
	hrs.				*mg.*	
	0	10	0	0	18.0	0
	0	10	10	0	18.2	0
(1) 37° C.	20	10	10	20	11.1	38.6
(2) 37° C.	20	10	10	20	9.1	49.7
(3) 37° C.	20	10	10	40	7.4	59.1
(4) 37° C.	20	10	0	40	5.3	70.7
(5) 37° C.	44	10	10	20	11.0	34.3
(6) 37° C.	44	10	0	20	12.0	33.7
	0	10	10	20	12.0	33.7

(1) (2) (3) and (4) were evaporated to dryness with acetic acid to expel H_2O_2 and then autoclaved with N. HCl. to convert creatine to creatinine.

(5) and (6) were evaporated to dryness after the addition of 2 cc. 10% HCl.

THE ELIMINATION OF PARENTERALLY ADMINISTERED CREATINE IN PHLORIDZIN DIABETES

Experiment F.—A female dog weighing 9 kilos served as the subject for this experiment. The urine was quantitatively collected by means of a catheter. On the third and subsequent days the dog refused all food. Subcutaneous injections of 1.2 gm. of phloridzin were made daily.

TABLE VII

THE RECOVERY OF INJECTED CREATINE IN PHLORIDZIN DIABETES

Day	Total Nitrogen	Creatinine	Creatine	Creatine Recovered	Sugar	D : N Ratio
	gm.	*gm.*	*mg.*	*per cent*	*gm.*	
1	15.85	313	217		10.66	0.67
2	15.62	...	...		10.72	0.68
3	9.21	345	535		8.31	0.90
4	10.36	379	606		15.69	1.51
5*	9.24	408	1207	60	28.60	3.09
6	7.50	241	610		27.00	3.60
7	6.27	209	724		20.00	3.19
8*	6.22	284	1376		22.00	3.54
9	6.33	177	878	65	23.25	3.67

*Injected 1 gm. of creatine.

THE EFFECT OF VARIATIONS IN THE RATIO OF FAT AND CARBOHYDRATES IN THE DIET ON THE CREATINE OUTPUT AND FATE OF INGESTED CREATINE IN CASES OF WELL ESTABLISHED CREATINURIA

Case I.—V. Nord., white, male, 6 years of age, weight 18 kilos, entered the State University of Iowa Hospital on June 7, 1922.

The only entrance complaint was a peculiar irregularity of gait. The diagnosis was progressive pseudo hypertrophic muscular dystrophy. Examination showed no isolated paralysis but a general weakness of all extensor muscles. When the patient was placed on the floor he got up in the typical fashion of muscular dystrophy cases. The patient was observed for three weeks in the metabolism unit. He was not confined to bed at any time during this study. The metabolic findings are given in Table VIII.

Throughout the observations the diet was practically purine and creatine free.* In the first period, days 1 to 6 inclusive, the diet consisted of 46 gm. of protein, 181 gm. of carbohydrate and 74 gm. of fat. On the fifth day he received 0.200 gm. of creatine by mouth. In the second period, days 15 to 22 inclusive, the energy value of the diet was 1,600 calories and consisted of 51 gm. of protein, 107 gm. of fat and 108 gm. of carbohydrate. The articles making up the diet included milk, egg, bread, butter, macaroni, cheese, sugar, pineapple, orange, tomato, and lettuce. On the nineteenth day he received 0.200 gm. creatine by mouth. In order to study the effect of feeding the incomplete protein gelatin, 30 gm. of gelatin were substituted for corresponding amounts of the mixed protein of the diet on days 21 and 22. The gelatin was consumed in one meal on the 21st day but emesis followed its administration on the following day. No attempt was made to substitute for this protein on this day. In the third period, days 24 to 29 inclusive, the total calories and protein was kept the same as in the preceding period but the patient consumed 124 gm. of fat and 73 gm. of carbohydrate. This diet included milk, bread, butter, egg, potato, cheese, tomato, lettuce, corn, orange and grape fruit. On the twenty-eighth day he again received 0.200 gm. of creatine by mouth. It had been planned to further decrease the ratio of carbohydrate to fat in the diet in subsequent periods but on the beginning of the twenty-ninth day definite symptoms of a beginning acidosis were in evidence. Emesis was frequent and the patient could not keep food or water on his stomach. Acetone was detected in the breath. The urine gave a strong reaction for acetone and diacetic acid. The respiratory rate was 26 per minute, pulse 100, and temperature 101° F. The special diet was discontinued and sweetened orange juice administered at various intervals. On the following day the patient had apparently recovered and was discharged. Owing to the difficulty in drawing blood we were unable to determine the CO_2 combining power of the plasma during the acidosis period.

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*I am indebted to Miss Lela Booker for preparation of the diets.

TABLE VIII

THE EFFECT OF VARIATIONS IN THE RATIO OF FAT AND CARBOHYDRATE ON THE CREATINE OUTPUT AND FATE OF INGESTED CREATINE

Day	Vol.	Total Nitrogen	Creatinine	Creatine	Amino Acid Nitrogen	Acetone	Protein	Carbohydrate	Fat
	cc.	*gm.*	*mg.*	*mg.*	*mg.*		*gm.*	*gm.*	*gm.*
1	1100	4.72	129	146	54	—	46	181	74
2	810	5.09	135	147	53	—	46	181	74
3	1130	5.34	139	177	54	—	46	181	74
4	940	5.23	124	154	58	—	46	181	74
Avg.	995	5.09	132	156	55				
5‡	1040	6.08	132	255	48		46	181	74
6	1060	5.72	131	126	60		46	181	74
15	1080	6.08	128	200	55	—	51	108	107
16	890	6.11	134	189	44	—	51	108	107
17	730	6.88	124	191	46	—	51	108	107
18	660	6.54	123	160	47	—	51	108	107
Avg.	840	6.40	127	185	48	1			
19‡	780	6.57	131	275	42	+	51	108	107
20	630	6.42	119	171	50	—	51	108	107
21*	720	7.13	120	304	93	+	51	108	107
22*†	880	5.17	120	299	68	+++	51	108	107
24	620	5.12	123	160	48	++++	51	73	124
25	700	5.64	125	160	34	++	51	73	124
26	750	5.84	127	148	43	++	51	73	124
Avg.	690	5.53	125	156	42		51	73	124
27‡	730	6.00	122	262	49	++	51	73	124
28	1010	4.80	142	171	38	++++	51	73	124

*30 gm. gelatin substituted for diet protein.

†Emesis following gelatin.

‡.200 gm. creatine by mouth.

Case II.—S. Ian., white, female, 15 years of age, weight 55 kilos, entered the State University of Iowa Hospital on January 10, 1919, for treatment for infantile paralysis contracted at the age of 3 months. The physical examination revealed no evidence of metabolic disturbances of any sort. The patient walked with a valgus deformity of the right foot and varus deformity of the left. There was a shortening of one and one-half inch of the left leg as compared with the right. The patient was confined to a wheel chair for the period during which the observations reported in this paper were made. The metabolic findings are given in Table IX.

Throughout the observations the diet was practically purine and creatine-free. In the first period, days 1 to 5 inclusive, the diet consisted of 76 gm. of protein, 160 gm. of fat and 163 gm. of carbohydrate. On the fourth day 0.640 gm. of creatine was given by mouth. In the second period, days 6 to 9 inclusive, the diet contained 76 gm. of protein, 202 gm. of fat and 121 gm. of carbohydrate. On the eighth day she received 0.640 gm. creatine by mouth. The patient showed no signs of acidosis and tests for acetone in the urine showed only traces present in the second period. Marked climatic changes are largely responsible for the marked variations in the volume of the daily output of urine. It was considered inadvisable to increase the quantity of fat in the diet and the patient was retained for further study on the effect of gelatin ingestion on the constituents of the urine.

TABLE IX

THE EFFECT OF VARIATIONS OF FAT AND CARBOHYDRATES ON THE CREATINE OUTPUT AND FATE OF INGESTED CREATINE

Day	Vol.	Total Nitrogen	Creatinine	Creatine	Acetone	Protein	Fat	Carbo.
	cc.	*gm.*	*mg.*	*mg.*		*gm.*	*gm.*	*gm.*
1	1080	9.42	623	433	—	76	160	163
2	1220	10.52	621	447	—	76	160	163
3	880	9.68	551	633	—	76	160	163
Avg.		9.87	598	504				
4*	1500	10.31	626	930	—	76	160	163
5	830	8.16	635	514	—	76	160	163
6	1930	11.42	666	490	tr.	76	202	121
7	1360	8.38	502	491	tr.	76	202	121
Avg.			582	491				
8*	1580	10.04	575	987	tr.	76	202	121
9	1500	8.82	580	534	tr.	76	202	121

*0.640 gm. creatine by mouth.

DISCUSSION

The experimental work presented in this investigation lends no support to the idea that creatine formation and subsequent elimination is related to carbohydrate metabolism, analogous to the explanation offered by Shaffer for the occurrence of acetone bodies in the urine.

Experiments on the influence of glucose on the oxidation of creatine by hydrogen peroxide in alkaline solution are much harder to control than those reported by Shaffer for acetoacetic acid. Creatine itself is slowly destroyed in even a weakly alkaline solution. Furthermore the addition of acid does not seem to entirely check the oxidation as it does in the case of acetoacetic acid. Another troublesome factor encountered is that all of the hydrogen peroxide must be removed from the solution before a determination of creatine as creatinine can be made by the Jaffe reaction, in as much as the presence of hydrogen peroxide lessens the color value obtained. On the whole the experiments reported in this paper tend to show that creatine is oxidized by hydrogen peroxide very nearly as readily without the addition of glucose as with. No such accelerating influence was found that is comparable to the oxidation of acetoacetic acid.

The experiments reported on the effect of pancreas extract on the sugar content and the creatine and creatinine balance of autolyzing muscle show that a preparation of the pancreas, prepared according to a method which has been shown to yield an active preparation as judged by its inhibiting effect on hyperglycemia and glycosuria, has no effect on these substances under the experimental conditions. This confirms the work of Levene and Meyer on the inability of panrceas extract to cause an oxidation of sugar when added to autolyzing muscle. These authors used a boiled pancreas extract and boiling has been shown to be destructive to this extract. Under the conditions of the experiment pancreas extract had no influence on the destruction of creatine or its conversion to creatinine.

The results of the experiments on the influence of kidney extract on the conversion of creatine to creatinine show that under the experimental conditions the kidney extract has no greater power to bring about this conversion than does muscle extract. They lend no support to the theory advanced by Behre and Benedict that the kidney is the seat of the transformation of creatine to creatinine.

The effect of variations in the ratio of fat and carbohydrate in the diets upon the creatine output and fate of ingested creatine in the two cases of creatinuria studied are interpreted as evidence that creatine formation and subsequent elimination are not dependent on carbohydrate metabolism analogous to a ketogenic and anti-ketogenic balance. In case I the ratio of fat and carbohydrate in the diet was such as to evoke marked clinical symptoms of acidosis. Analysis of the urine, however, shows that the creatine output is not influenced and that ingested creatine is eliminated in approximately the same amount as on a diet much higher in carbohydrate and lower in fat. Similar results were encountered in Case II although this patient showed no symptoms of acidosis on a diet in which the ratio of fat to carbohydrate was much higher than in the preceding case. However, it is to be noted that in these cases no increased output of urinary nitrogen accompanied the high fat periods. The experiment reported on creatine elimination in phloridzin diabetes is significant in that it shows that a dog, rendered completely diabetic and having Lusk's fatal ratio of 1 : 3.65, fails to eliminate injected creatine in amounts greater than in other conditions of well established creatinuria with little or no derangement of carbohydrate metabolism. This finding is not in harmony with the theory advanced by Benedict and Osterberg that normally large amounts of creatine are formed in the body but appear in the urine in conditions of carbohydrate deficiency. In the case investigated the animal is assumed to be unable to utilize any carbohydrate and yet injected creatine fails to reappear in the urine in anything like quantitative amounts.

SUMMARY

1. No evidence was found that the formation and subsequent elimination of creatine is related to carbohydrate metabolism, analogous to that reported for acetone formation and elimination by Shaffer. Variations in the ratio of fat and carbohydrate in the diets were without influence on the creatine output in the two cases studied.
2. A dog, rendered completely diabetic with phloridzin, fails to eliminate considerable amounts of injected creatine.
3. The addition of pancreas extract is without effect on the creatine and creatinine balance of autolyzing muscle.
4. Under the conditions of the experiments kidney extract has no greater power to bring about a conversion of creatine to creatinine than muscle extract.

BIBLIOGRAPHY

1. Liebig, J., *Liebig's Annalen,* 1847, LXII, 257.
2. Pettenkofer, M., *Liebig's Annalen,* 1844, LII, 97.
3. Folin, O., *Z. Physiol. Chem.,* 1904, XLI, 223.
4. Folin, O., *Amer. J. Physiol.,* 1905, XIII, 66.
5. Mellanby, E., *Amer. J. Physiol.,* 1908, XXXVI, 447.
6. Myers, V. C., and Fine, M. S., *J. Biol. Chem.,* 1913, XIV, 9.
7. Myers, V. C., and Fine, M. S., *J. Biol. Chem.,* 1915, XXI, 583.
8. Hammett, F. S., *J. Biol. Chem.,* 1921, XLVIII, 133, 143.
9. Behre, J. A., and Benedict, S. R., *J. Biol. Chem.,* 1922, LII, 11.
10. Denis, W., *J. Biol. Chem.,* 1917, XXX, 47.
11. Folin, O., and Denis, W., *J. Biol. Chem.,* 1912, XI, 253.
12. Denis, W., and Krammer, J. G., *J. Biol. Chem.,* 1917, XXX, 189.
13. Gamble, J. L., and Goldschmidt, S., *J. Biol. Chem.,* 1919, XL, 199.
14. Rose, W. C., Dimmett, J. S., and Bartlette, H. L., *J. Biol. Chem.,* 1918, XXXIV, 601.
15. Denis, W., and Minot, A. S., *J. Biol. Chem.,* 1918, XXXVII, 255.
16. Gross, E. G., and Steenbock, H., *J. Biol. Chem.,* 1921, XLVII, 45.
17. Gibson, R. B., and Martin, F. T., *J. Biol. Chem.,* 1921, XLIX, 319.
18. Cathcart, E. P., *J. Physiol.,* 1909-10, XXXIX, 311.
19. Mendel, L. B., and Rose, W. C., *J. Biol. Chem.,* 1911-12, X, 213.
20. Rose, W. C., *J. Biol. Chem.,* 1911, X, 265.
21. Rose, W. C., *J. Biol. Chem.,* 1916, XXVI, 331.
22. Myers, V. C., and Fine, M. S., *J. Biol. Chem.,* 1913, XV, 305.
23. Underhill, F. P., and Baumann, E. J., *J. Biol. Chem.,* 1916, XXVII, 151.
24. Benedict, S. R., and Osterberg, E., *J. Biol. Chem.,* 1914, XVIII, 195.
25. Krause, R. A., and Cramer, W. J., *J. Physiol.,* 1910, XL, 61.
26. Underhill, F. P., *J. Biol. Chem.,* 1916, XXVII, 127, 141.
27. Sawyer, M., Stevens, F. A., and Baumann, L., *Am. J. Dis. Child.,* 1918, XV, 1.
28. Shaffer, P. A., *J. Biol. Chem.,* 1921, XLVII, 433, 449.
29. Shaffer, P. A., *J. Biol. Chem.,* 1921, XLIX, 143.
30. Shaffer, P. A., *J. Biol. Chem.,* 1922, L, 26.
31. Levene, P. A., and Kristeller, L., *Am. J. Physiol.,* 1909, XXIV, 45.
32. Powis, F., and Raper, H. S., *Biochem. J.,* 1916, X, 363.
33. Banting, F. G., and Best, C. H., *Jour. Lab. and Clin. Med.,* 1922, VII, 464.
34. Levene, P. A., and Meyer, G. M., *J. Biol. Chem.,* 1910, IX, 67.
35. Folin, O., and Wu, H., *J. Biol. Chem.,* 1919, XXXVIII, 81.
36. Hammett, F. S., *J. Biol. Chem.,* 1921, XLVIII, 127.

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